NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

SODIUM NITRITE

(CAS NO. 7632-00-0)

IN F344/N RATS AND B6C3F$_1$ MICE

(DRINKING WATER STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

May 2001

NTP TR 495

NIH Publication No. 01-3954

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

Details about ongoing and completed NTP studies are available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov. Abstracts of all NTP Technical Reports and full versions of the most recent reports and other publications are available from the NIEHS' Environmental Health Information Service (EHIS) http://ehis.niehs.nih.gov (800-315-3010 or 919-541-3841). In addition, printed copies of these reports are available from EHIS as supplies last. A listing of all the NTP reports printed since 1982 appears on the inside back cover.

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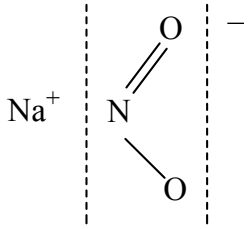
CONTENTS

ABSTRACT



SODIUM NITRITE

CAS No. 7632-00-0

Chemical Formula: $NaNO_2$ Molecular Weight: 69.00

Synonyms: Diazotizing salts; nitrous acid, sodium salt
Trade names: Anti-Rust, Erinitrit, Filmerine

Sodium nitrite is used as a color fixative and preservative in meats and fish. It is also used in manufacturing diazo dyes, nitroso compounds, and other organic compounds; in dyeing and printing textile fabrics and bleaching fibers; in photography; as a laboratory reagent and a corrosion inhibitor; in metal coatings for phosphatizing and detinning; and in the manufacture of rubber chemicals. Sodium nitrite also has been used in human and veterinary medicine as a vasodilator, a bronchial dilator, an intestinal relaxant, and an antidote for cyanide poisoning. Sodium nitrite was nominated by the FDA for toxicity and carcinogenesis studies based on its widespread use in foods. Male and female F344/N rats and $B6C3F_1$ mice were exposed to sodium nitrite (99% pure) in drinking water for 14 weeks or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, rat and mouse bone marrow, and mouse peripheral blood.

14-WEEK STUDY IN RATS

Groups of 10 male and 10 female rats were exposed to 0, 375, 750, 1,500, 3,000, or 5,000 ppm sodium nitrite (equivalent to average daily doses of approximately 30, 55, 115, 200, or 310 mg sodium nitrite/kg body weight to males and 40, 80, 130, 225, or 345 mg/kg to females) in drinking water for 14 weeks. Clinical pathology study groups of 15 male and 15 female rats were exposed to the same concentrations for 70 or 71 days. One female exposed to 3,000 ppm died before the end of the study. Body weights of males exposed to 3,000 or 5,000 ppm and females exposed to 5,000 ppm were significantly less than those of the controls. Water consumption by 5,000 ppm males and 3,000 and 5,000 ppm females was less than that by the controls at weeks 2 and 14. Clinical findings related to sodium nitrite exposure included brown discoloration in the eyes and cyanosis of the mouth, tongue, ears, and feet of males exposed to 3,000 or 5,000 ppm and of females exposed to 1,500 ppm or greater. Reticulocyte counts were increased in males and females exposed to 3,000 or 5,000 ppm. The erythron was decreased on day 19 but increased by week 14 in males and females exposed to 5,000 ppm. Methemoglobin concentrations were elevated in almost all exposed groups throughout the 14 week study; a no-observed-adverse-effect level was not achieved. The relative kidney and spleen weights of males and females exposed to 3,000 or 5,000 ppm were significantly greater than those of the controls. Sperm motility in 1,500 and 5,000 ppm males was significantly decreased. Increased erythropoietic

activity in the bone marrow of exposed males and females was observed. The incidences of squamous cell hyperplasia of the forestomach in 5,000 ppm males and females were significantly increased.

14-Week Study in Mice

Groups of 10 male and 10 female B6C3F$_1$ mice were exposed to 0, 375, 750, 1,500, 3,000, or 5,000 ppm sodium nitrite (equivalent to average daily doses of approximately 90, 190, 345, 750, or 990 mg/kg to males and 120, 240, 445, 840, or 1,230 mg/kg to females) in drinking water for 14 weeks. Body weights of males exposed to 5,000 ppm were significantly less than those of the controls. Water consumption by males exposed to 1,500 ppm or greater was slightly less than that by the controls at week 13. Relative spleen weights of 3,000 and 5,000 ppm males and absolute and relative heart, kidney, liver, and spleen weights of females exposed to 3,000 or 5,000 ppm were greater than those of the control groups. Sperm motility was decreased in 5,000 ppm males, and the estrous cycles of 1,500 and 5,000 ppm females were significantly longer than in the controls. There were increased incidences of squamous cell hyperplasia of the forestomach in 5,000 ppm males and females, extramedullary hematopoiesis of the spleen in 3,000 and 5,000 ppm males and 1,500 ppm or greater females, and degeneration of the testis in 3,000 and 5,000 ppm males.

2-Year Study in Rats

Groups of 50 male and 50 female rats were exposed to 0, 750, 1,500, or 3,000 ppm sodium nitrite (equivalent to average daily doses of approximately 35, 70, or 130 mg/kg to males and 40, 80, or 150 mg/kg to females) in drinking water for 2 years. For toxicokinetic studies of plasma nitrite and blood methemoglobin, 10 male and 10 female special study rats were exposed to the same concentrations for 12 months. Survival of exposed groups was similar to that of the controls. Mean body weights of males and females exposed to 3,000 ppm were less than those of the controls throughout the study. Water consumption by males and females exposed to 3,000 ppm was less than that by the controls throughout the study, and that by the other exposed groups was generally less after week 14.

The incidences of hyperplasia of the forestomach epithelium in males and females exposed to 3,000 ppm were significantly greater than those in the control groups. The incidence of fibroadenoma of the mammary gland was significantly increased in females exposed to 1,500 ppm, and the incidences of multiple fibroadenoma were increased in 750 ppm and 1,500 ppm females; however, these neoplasms occur with a high background incidence, and no increase was seen in the 3,000 ppm group. The incidences of mononuclear cell leukemia were significantly decreased in males and females exposed to 1,500 or 3,000 ppm.

2-Year Study in Mice

Groups of 50 male and 50 female B6C3F$_1$ mice were exposed to 0, 750, 1,500, or 3,000 ppm sodium nitrite (equivalent to average daily doses of approximately 60, 120, or 220 mg/kg to males and 45, 90, or 165 mg/kg to females) in drinking water for 2 years. Survival of exposed groups was similar to that of the controls; mean body weights of 3,000 ppm females were less than those of the controls throughout the study. Exposed groups generally consumed less water than the control groups.

The incidences of squamous cell papilloma or carcinoma (combined) in the forestomach of female mice occurred with a positive trend. The incidence of hyperplasia of the glandular stomach epithelium was significantly greater in 3,000 ppm males than in the controls.

Genetic Toxicology

Sodium nitrite was mutagenic in *Salmonella typhimurium* strain TA100, with and without Aroclor 1254-induced hamster and rat liver S9 enzymes; no mutagenicity was observed in strain TA98. Results of acute bone marrow micronucleus tests with sodium nitrite in male rats and mice by intraperitoneal injection were negative. In addition, a peripheral blood micro nucleus assay conducted with mice from the 14-week study gave negative results.

Conclusions

Under the conditions of this 2-year drinking water study, there was *no evidence of carcinogenic activity** of sodium nitrite in male or female F344/N rats

exposed to 750, 1,500, or 3,000 ppm. There was *no evidence of carcinogenic activity* of sodium nitrite in male $B6C3F_1$ mice exposed to 750, 1,500, or 3,000 ppm. There was *equivocal evidence of carcinogenic activity* of sodium nitrite in female $B6C3F_1$ mice based on the positive trend in the incidences of squamous cell papilloma or carcinoma (combined) of the forestomach.

Exposure to sodium nitrite in drinking water resulted in increased incidences of epithelial hyperplasia in the forestomach of male and female rats and in the glandular stomach of male mice.

Decreased incidences of mononuclear cell leukemia occurred in male and female rats.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report is on page 13.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Sodium Nitrite

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Concentrations in drinking water	0, 750, 1,500, or 3,000 ppm	0, 750, 1,500, or 3,000 ppm	0, 750, 1,500, or 3,000 ppm	0, 750, 1,500, or 3,000 ppm
Body weights	3,000 ppm group less than the control group	3,000 ppm group less than the control group	Exposed groups similar to the control group	3,000 ppm group less than the control group
Survival rates	29/50, 38/50, 36/50, 36/50	33/50, 31/50, 36/50, 33/50	39/50, 45/50, 42/50, 39/50	40/50, 34/50, 37/50, 41/50
Nonneoplastic effects	Forestomach: epithelial hyperplasia (12/50, 9/50, 10/50, 44/50)	Forestomach: epithelial hyperplasia (8/50, 6/50, 8/50, 40/50)	Glandular stomach: epithelial hyperplasia (0/50, 0/50, 2/50, 10/50)	None
Neoplastic effects	None	None	None	None
Uncertain findings	None	None	None	Forestomach: squamous cell papilloma or carcinoma (1/50, 0/50, 1/50, 5/50)
Decreased incidences	Mononuclear cell leukemia: (17/50, 12/50, 7/50, 3/50)	Mononuclear cell leukemia: (15/50, 10/50, 1/50, 1/50)	None	None
Level of evidence of carcinogenic activity	No evidence	No evidence	No evidence	Equivocal evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:	Positive in strain TA100 with and without S9; negative in strain TA98			
Micronucleated erythrocytes				
Male rat bone marrow *in vivo*:	Negative			
Male mouse bone marrow *in vivo*:	Negative			
Male and female mouse peripheral blood *in vivo*:	Negative			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on sodium nitrite on 18 May 2000 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 18 May 2000, the draft Technical Report on the toxicology and carcinogenesis studies of sodium nitrite received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. P.C. Chan, NIEHS, introduced the toxicology and carcinogenesis studies of sodium nitrite by describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic lesions in female mice, possible compound-related neoplastic lesions in female rats, and compound-related nonneoplastic lesions in male and female rats and male mice. The proposed conclusions were *no evidence of carcinogenic activity* in male F344/N rats or male B6C3F$_1$ mice, *equivocal evidence of carcinogenic activity* in female F344/N rats, and *some evidence of carcinogenic activity* in female B6C3F$_1$ mice.

Dr. F. Ye, NIEHS, presented statistical analyses and information on the toxicokinetic modeling of nitrite absorption and elimination and methemoglobinemia in rats and mice. The objectives of the analyses were to study the relationship between nitrite and methemoglobinemia by developing a toxicokinetic model to characterize the nitrite-induced methemoglobin process and to compare net absorption and elimination rates of nitrite from plasma. The studies found that nitrite is rapidly absorbed after oral exposure and may depend on dose level. The studies also found that overall clearance of nitrite may depend on species, route, and dose, and nitrite is rapidly cleared from plasma because it causes oxidation to methemoglobin by binding to hemoglobin. Dr. Ye further concluded that reduction of hemoglobin to its ferrous form is sensitive to basal methemoglobin reductase activity, strong binding of nitrite to methemoglobin, and the autocatalytic cycle.

Dr. Hecht, a principal reviewer, agreed with the proposed conclusions in regard to male rats but disagreed with the proposed conclusions in regard to female mice, for which he stated the data only supported equivocal evidence of carcinogenic activity. Dr. Hecht also stated that the NTP had ignored literature on endogenous formation of nitrosamines and that the literature should be updated and expanded.

Dr. Bus, the second principal reviewer, agreed with the proposed conclusions for male rats and mice but disagreed with the proposed conclusions for female rats and mice. For female rats, he argued that the response in the mammary glands was not exposure concentration dependent, occurred in the presence of high concurrent and historical control incidences of fibroadema, and was not accompanied by increased incidences of carcinomas, thus supporting no evidence of carcinogenic activity. Dr. J.R. Bucher, NIEHS, stated the incidence in the mid-exposure group was the highest seen, and the finding could not be dismissed. Dr. Bus emphasized that the increased incidence of forestomach squamous cell papilloma or carcinoma (combined) in 3,000 ppm female mice was not significantly different from that in the controls, and that only the trend test showed statistical significance, thus supporting equivocal evidence of carcinogenic activity. Dr. Chan agreed that the level of evidence in female mice could be debated. Due to the positive trend in the incidences of squamous cell papillomas and carcinomas (combined) and because the incidence in the 3,000 ppm females exceeded the historical range for NTP controls on NTP-2000 diet, he concluded the evidence supported some evidence of carcinogenic activity. Also, he found the two carcinomas hard to ignore.

Dr. Chatman, the third principal reviewer, disagreed with the proposed conclusions for female rats and mice. Dr. Chatman agreed with Dr. Bus that the level of evidence should be no evidence of carcinogenic activity in female rats and equivocal evidence of carcinogenic activity in female mice. Dr. Chatman questioned the use of drinking water rather than dosed feed as the route of administration.

Dr. J.R. Hailey, NIEHS, addressed a comment by Dr. Bus and by Dr. G. Williams, New York Medical College, concerning incidences of gastrointestinal neoplasms in mice on the NTP-2000 diet compared with the NIH-07 diet. Dr. Hailey stated there was an increase noted in the incidences of intestinal neoplasms but not in forestomach neoplasms. Dr. Bailer suggested that significant negative trends in the

incidences of neoplasms such as mononuclear cell leukemia in male and female rats warranted more attention than given in the report.

Dr. L.L. Borchert, Director of Research (Retired), Oscar Mayer Foods Corporation and Adjunct Professor, Meat Science and Muscle Biology, University of Wisconsin-Madison, representing the American Meat Science Association, provided historical background on the food industry's need to use sodium nitrite and the safety of sodium nitrite as a food additive. He stated the United States Department of Agriculture and its predecessors have permitted the use of sodium nitrite in processed meat products since 1925, when it was proven safe for human consumption. He said the industry welcomed the use of sodium nitrite because it accelerated the curing process from days and weeks to hours. He stated that in order to meet safety concerns arising in the 1970s, the food industry eliminated sodium nitrate, reduced usage levels of sodium nitrite, incorporated sodium ascorbate into all processed meat products, and monitored nitrosamine formation in fried bacon. He noted a national survey from 1996 which showed that processed meats in the United States contain about 10 ppm of sodium nitrite and no sodium nitrate.

Dr. D. Archer, Food Science and Human Nutrition Department, University of Florida, representing the Food Safety Advisory Committee of the American Meat Institute Foundation, noted some of the positive aspects of having sodium nitrite in the food supply. First, he stressed the positive physiologic role of nitric oxide and its metabolites, including nitrite. Second, he noted that nitrite is a physiologically endogenous compound. Third, he emphasized the profound effect of sodium nitrite in preventing growth of and toxin production by *Clostridium botulinum*. Fourth, he remarked on the growth retardant role that sodium nitrite plays on food pathogens as a bactericidal agent in conjunction with stomach acid.

Dr. G. Williams, New York Medical College, representing the American Meat Institute Foundation, asserted that due to several reasons, the small incidence of forestomach neoplasms seen in 3,000 ppm female mice at the end of the study is not attributable to sodium nitrite. He commented that the high pH of the female mouse forestomach and sodium nitrite solutions used were not conducive to formation of DNA-deaminating nitrous acid or of carcinogenic N-nitroso compounds, and there was no evidence for this reaction. Also, he noted that the genetic toxicology database for sodium nitrite supports the view that it is not an *in vivo* genotoxic agent. Dr. Williams further stated that the new NTP-2000 diet appears to facilitate the development of more spontaneous gastrointestinal neoplasms than the NIH-07 diet, and sodium nitrite has not been shown to be a forestomach carcinogen in rats in this bioassay or in the scientific literature.

Dr. Hecht moved that the Technical Report on sodium nitrite be accepted with revisions discussed including changes in the conclusions for female rats and mice. The proposed revised conclusions were, for male and female rats and male mice, *no evidence of carcinogenic activity* and for female mice, *equivocal evidence of carcinogenic activity*. Dr. Bus seconded the motion. The motion was accepted unanimously with six yes votes.